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Marinobufagenin and left ventricular mass in young adults: The African-PREDICT study

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Abstract

Background: The endogenous steroidal inhibitor of sodium–potassium-dependent adenosine triphosphate and natriuretic hormone, marinobufagenin, plays a physiological role in ionic homeostasis. Animal models suggest that elevated marinobufagenin adversely associates with cardiac and renal, structural and functional alterations. It remains uncertain whether marinobufagenin relates to the early stages of target organ damage development, especially in young adults without cardiovascular disease. We therefore explored whether elevated 24-hour urinary marinobufagenin excretion was related to indices of subclinical target organ damage in young healthy adults.

Design: This cross-sectional study included 711 participants from the African-PREDICT study (black 51%, men 42%, 24.8 ± 3.02 years).

Methods: We assessed cardiac geometry and function by two-dimensional echocardiography and pulse wave Doppler imaging. 24-Hour urinary marinobufagenin and sodium excretion were measured, and the estimated glomerular filtration rate determined.

Results: Across marinobufagenin excretion quartiles, left ventricular mass ($P < 0.001$), end diastolic volume ($P < 0.001$), stroke volume ($P = 0.004$) and sodium excretion ($P < 0.001$) were higher within the fourth compared with the first quartile. Partial regression analyses indicated that left ventricular mass ($r = 0.08$, $P = 0.043$), end diastolic volume ($r = 0.10$, $P = 0.010$) and stroke volume ($r = 0.09$, $P = 0.022$) were positively related to marinobufagenin excretion. In multivariate-adjusted regression analysis, left ventricular mass associated positively with marinobufagenin

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Author contribution

MS, WS, RK, OVF and AES contributed to the concept and design. MS, WS, RK, WW, OVF and AES contributed to the acquisition, analyses and interpretation of data. MS drafted the manuscript. WS, RK, WW, OVF and AES critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work ensuring integrity and accuracy.

Declaration of conflicting interests

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excretion only in the highest marinobufagenin excretion quartile (adjusted $R^2 = 0.20$; $\beta = 0.15$; $P = 0.043$). This relationship between left ventricular mass and marinobufagenin excretion was evident in women (adjusted $R^2 = 0.06$; $\beta = 0.127$; $P = 0.015$) but not in men (adjusted $R^2 = 0.06$; $\beta = 0.007$; $P = 0.92$).

Conclusions: Left ventricular mass positively and independently associates with marinobufagenin excretion in young healthy adults with excessively high marinobufagenin excretion. Women may be more sensitive to the effects of marinobufagenin on early structural cardiac changes.

Keywords

Apparently healthy; left ventricular mass index; marinobufagenin; young adults

Introduction

Mammalian cardiotonic steroids include the endogenous bufadienolide marinobufagenin (MBG), which is synthesised by the adrenal cortex¹ in response to sodium-induced plasma volume expansion.² In accordance, we have previously indicated a strong correlation between estimated salt intake and MBG in young adults.³ Apart from MBG's primary natriuretic function via the classic ionic pathway whereby renal $\alpha 1$ -sodium-potassium-dependent adenosine triphosphate (Na^+/K^+ -ATPase) activity is inhibited, MBG additionally induces pro-fibrotic signalling through the Na^+/K^+ -ATPase in cardiovascular tissue.^{4,5} This effect of MBG on the cellular signalling function of Na^+/K^+ -ATPase is proposed to play an important role in disease development.⁴

Increasing evidence demonstrates the deleterious role of MBG particularly on cardiac^{5–8} and renal^{9,10} structure and function, implicating MBG in the pathogenesis of several conditions such as preeclampsia,^{11,12} uraemic cardiomyopathy^{5,6} and heart failure.^{7,13} Accordingly, elevated levels of MBG were shown to promote cardiac fibrosis^{5,6} and hypertrophy⁵ while also being associated with impaired left ventricular relaxation.^{5,8} Apart from cardiac effects, MBG promotes renal fibrosis⁹ and was shown to be elevated in patients with chronic kidney disease.¹⁴

Studies pertaining to MBG and the effect thereof on target organ damage (TOD) were performed in animals^{5,9} or human cohorts with present pathology such as heart failure (aged 58 ± 13 years),⁷ chronic kidney disease (aged 52.8 ± 3.7 years)¹⁴ and renal artery stenosis (aged 70.5 ± 1.3 years).¹⁰ Thus it is not yet clear whether MBG might be related to the initial stages of TOD development in young healthy adults, especially those previously shown to consume high amounts of salt,¹⁵ with resultant elevated MBG levels.³ It is of particular interest to demonstrate a possible relationship in this young population without overt cardiovascular disease (CVD), which should contribute to our understanding of the early pathophysiological role of MBG in CVD development. We therefore explored whether elevated levels of 24-hour urinary MBG excretion are related to indices of subclinical TOD in a young apparently healthy adult population aged 20–30 years.

Contingent upon a relationship between MBG and indices of subclinical TOD in this young population, our study would support further investigation into MBG as a potential marker of early cardiovascular risk. Moreover, evidence of a relationship between MBG and indices of subclinical TOD in this population consuming a habitual high salt diet,¹⁵ would endorse the implementation of global preventive salt reduction legislation.

Methods

Study design and participant recruitment

This cross-sectional study included data of the first 711 consecutively enrolled participants (black 51%, men 42%) from the African Prospective study on the Early Detection and Identification of Cardiovascular disease and Hypertension (African-PREDICT), with complete 24-hour urinary data.

The study recruited young apparently healthy black and white adults (aged 20–30 years) from the North West Province of South Africa. Participants were recruited between 2012 and 2017. Community members, in proximity to the Potchefstroom area, were invited on a voluntary basis to participate in the initial health screening prior to inclusion in the study. The inclusion criteria were microalbuminuria less than 30 mg/ml and HIV uninfected. Participants were included based on office blood pressure less than 140/90 mmHg, which is in line with the 2013 guidelines set by the European Society of Hypertension and European Society of Cardiology.¹⁶ In addition, none of the participants were previously diagnosed with any chronic illness, or were using antihypertensive or chronic disease medication (self-reported). None of the women participating in this study were pregnant or lactating.

Eligible participants were invited back for further measurements at the hypertension research clinic on the North-West University campus. Participants arrived at 08:00 and were introduced to the research environment. Organisational procedures were explained prior to the start of measurements.

The African-PREDICT study protocol was approved by the health research ethics committee of the North-West University, and is registered at [ClinicalTrials.gov](https://clinicaltrials.gov) (no. NCT03292094). All procedures complied with institutional guidelines and the Declaration of Helsinki. Written informed consent was provided by each participant before participation in the study. Reporting of the study conforms to the STROBE statement along with references to the STROBE statement and the broader EQUATOR guidelines.¹⁷

Cardiovascular measurements

A single registered medical clinical technologist performed two-dimensional echocardiography using the General Electric Vivid E9 device (GE Vingmed Ultrasound A/S, Horten, Norway), and a 2.5–3.5 MHz transducer and a single ECG lead. Echocardiographic imaging was performed in accordance with standardised procedures outlined by the American Society of Echocardiography and the European Association of Cardiovascular Imaging.^{18–20} Measures including the left ventricular mass (LVM) and left ventricular volumes were determined using the biplane method, and defined by indexation to body surface area (BSA) or height. LVM was normalised for BSA (LVMi),²⁰ stroke volume for

height to the power of 2.04 (SVi) and cardiac output for height to the power of 1.83.²¹ End systolic volume (ESV) and end diastolic volume (EDV) were calculated using the Teichholz formula,²² and EDV subsequently indexed for height (EDVi). In addition, we determined indices of systolic function including fractional shortening (FS) and left ventricular ejection fraction ((EDV–ESV)/EDV).

Left ventricular filling was assessed by means of pulse wave Doppler imaging performed in the apical four-chamber view. Sample volumes were placed 1–3 mm between the tips of the mitral valve leaflets in parallel alignment to inflow. The peak velocities of early (E) and late (A) diastolic filling, the E/A ratio and the left atrial to aortic root ratio (LA:Ao) were measured as parameters of diastolic function. In addition, we determined myocardial tissue movement using tissue Doppler imaging in order to calculate the ratio between the transmitral peak E velocity and the early diastolic mitral annular velocity (E/é).

Biological sampling and biochemical analyses

Participants were requested not to eat or drink at least 8 hours prior to measurements. A trained research nurse collected early morning blood samples at approximately the same time every morning and before cardiovascular measurements began. Following blood sample collection using red top serum tubes and sodium fluoride plasma tubes, samples were immediately taken from the hypertension research clinic to the on-site temperature controlled laboratory. Samples prepared for serum were allowed to clot for 30 minutes at room temperature and were subsequently centrifuged for 30 minutes at 1602g at room temperature (Hettich Universal 320; Andreas Hettich GmbH & Co., Tuttlingen, Germany) and aliquoted into cryovials. Sodium fluoride plasma tubes were placed on ice, and centrifuged for 10 minutes at 2307g (Hettich Universal 32R, Andreas Hettich GmbH & Co., Tuttlingen, Germany). Aliquoted samples were stored in the on-site biofreezers controlled at –80°C, until analysis.

Serum C-reactive protein (CRP), high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides, γ -glutamyl transferase (GGT), creatinine and sodium, and plasma glucose were analysed using the Cobas Integra 400plus (Roche, Basel, Switzerland). In addition, serum interleukin-6 (IL-6) (high sensitivity Quantikine ELISA kit; R&D Systems, Minneapolis, USA) and cotinine (chemiluminescence method on the Immulite; Siemens, Erlangen, Germany) were measured.

The 24-hour urine sampling for this study, from which we measured sodium, potassium, albumin and creatinine (Cobas Integra 400plus; Roche, Basel Switzerland), has previously been described³ and follow standard protocols by the Pan American Health Organization/World Health Organization (PAHO/WHO).²³ Twenty-four-hour urinary MBG was additionally analysed with a solid-phase dissociation-enhanced lanthanide fluorescent immunoassay based on a mouse monoclonal 4G4 anti-MBG antibody.²⁴ We determined the estimated glomerular filtration rate (eGFR) using the CKD-EPI equation,²⁵ and determined fractional sodium excretion (FENa)²⁶ using the following formula:

$$\text{FENa} = \frac{(\text{urinary Na} * \text{plasma creatinine})}{(\text{plasma Na} * \text{urinary creatinine})} * 100$$

Additional methods can be seen in the online supplement.

Statistical analyses

We used Statistica version 13.2 (Dell Inc., Tulsa, Oklahoma, USA) for data analyses. Non-Gaussian distributed variables were logarithmically transformed. We performed analyses of covariance to determine differences in cardiac structure, function and renal function across increasing quartiles of MBG excretion while adjusting for age, sex and ethnicity. Pearson, partial and multiple regression analyses were performed to explore relationships of subclinical TOD with MBG excretion. We additionally performed multiple regression analyses within respective MBG excretion quartiles, where measures of TOD (relative wall thickness, LVMi, EDVi, SVi, cardiac index (COi), LA:Ao, E:A, E:é, FENa, eGFR or 24-hour urinary albumin) were included in separate models as dependent variables. While we considered several covariates for inclusion as possible independent variables, sex, age, waist:height (WHtR), 24-hour systolic blood pressure (SBP), HDL-cholesterol, CRP, GGT and glucose were ultimately included in the final models based on the strongest bivariate associations with indices of TOD and MBG excretion. Multiple regression models in which case the dependent variables were normalised for BSA or height, were not adjusted for WHtR. Also, multiple regression models with echocardiographic parameters as dependent variables were additionally adjusted for eGFR. Interaction testing was performed for sex and ethnicity, and Pearson, partial and multiple regression analyses were repeated accordingly. Missing data for dependent variables included LVMi ($n = 2$), relative wall thickness ($n = 2$), EDVi ($n = 2$), SVi ($n = 3$), COi ($n = 4$), ejection fraction ($n = 2$), FS ($n = 3$), LA:Ao ($n = 2$), E:A ($n = 5$), E:é ($n = 7$), FENa ($n = 37$), eGFR ($n = 22$), albumin ($n = 18$). Pearson and partial analyses were performed using pairwise deletion of data, while casewise deletion was used for multiple regression analyses.

Results

Participant characteristics

Table 1 presents the descriptive characteristics of this young adult population (aged 24.8 ± 3.02 years) by increasing quartiles of MBG excretion. We found that EDVi ($P = 0.003$), SVi ($P = 0.003$) and 24-hour SBP ($P = 0.001$) increased along increasing quartiles of MBG. Differences between quartiles 1 and 4 for EDVi ($P < 0.001$), SVi ($P = 0.004$) and 24-hour SBP ($P < 0.001$) were also noted after adjustment for age, sex and ethnicity. Similarly, LVMi was higher within the fourth quartile when compared with the first quartile of MBG excretion ($P < 0.001$). As expected, individuals with increased MBG had significantly higher 24-hour urinary volume, sodium, potassium and creatinine excretion.

Regression analyses

Unadjusted analyses indicated significant relationships between indices of subclinical TOD and MBG excretion (Figures 1 and 2). Once we adjusted for age, sex and ethnicity the relationships with LVMi ($r = 0.08$, $P = 0.043$), EDVi ($r = 0.10$, $P = 0.010$) and SVi ($r = 0.09$, $P = 0.022$) remained significant, but not eGFR ($r = 0.004$, $P = 0.92$). When we applied full adjustments in multivariate adjusted analyses in the total group, these relationships became borderline significant, namely with LVMi (adjusted $R^2 = 0.23$; SD $\beta = 0.066$; $P = 0.084$),

EDVi (adjusted $R^2 = 0.22$; SD $\beta = 0.068$; $P = 0.079$) and SVi (adjusted $R^2 = 0.07$; SD $\beta = 0.072$; $P = 0.089$) (see Supplementary Table 1).

However, as we noted marked differences in LVMi, EDVi, SVi and eGFR between the lowest and highest quartiles of MBG excretion in Table 1, we additionally investigated these relationships more thoroughly within respective quartiles. When performing single or partially adjusted linear regression analyses, we found no correlations of LVMi, EDVi, SVi or eGFR with MBG excretion in either quartile (see Supplementary Table 2). However, in multivariate adjusted regression analyses we found that LVMi demonstrated a positive tendency across increasing quartiles, and associated positively with MBG excretion in the highest MBG excretion quartile only (adjusted $R^2 = 0.20$; SD $\beta = 0.15$; $P = 0.043$; $N = 165$) (see Figure 2 and Supplementary Table 3).

Sensitivity analyses

Evident in this study was the high salt intake of the population, with more than 78% ($n = 557$) of the young adults consuming more than the recommended 5 g of salt per day. We have previously demonstrated a positive relationship between estimated sodium chloride (NaCl) intake and MBG excretion,³ also confirmed in this population ($r = 0.39$; $P < 0.001$). We therefore performed a sensitivity analysis to determine whether the association found in the highest quartile between LVMi and MBG excretion was independent of NaCl intake (see Supplementary Table 4). Forward stepwise multiple regression analysis indicated that this relationship was not confounded by NaCl as LVMi remained significantly related to MBG excretion (adjusted $R^2 = 0.21$; SD $\beta = 0.15$; $P = 0.041$) and NaCl did not enter the model.

Interaction of sex and ethnicity

Interaction testing for sex and ethnicity on the relationships between indices of subclinical TOD and MBG excretion or MBG excretion quartiles is presented in Supplementary Table 5. We found a significant interaction with sex only on the relationship of MBG excretion with SVi, COi and FENa, while no interaction existed with ethnicity. Therefore, Pearson, partial and multiple regression analyses were repeated in men and women, respectively (Supplementary Table 6).

Notably, the relationship between LVMi and MBG excretion was observed only in women ($N = 374$) (adjusted $R^2 = 0.06$; SD $\beta = 0.127$; $P = 0.015$), while the relationship of EDVi (adjusted $R^2 = 0.09$; SD $\beta = 0.128$; $P = 0.043$) and COi (adjusted $R^2 = 0.18$; SD $\beta = 0.119$; $P = 0.048$) with MBG excretion were predominant in men ($N = 252$).

Discussion

To our knowledge, our study is the first to investigate whether elevated levels of urinary MBG excretion relate to indices of subclinical TOD in young adults, free of detected cardiovascular or other chronic diseases. We found a positive, blood pressure independent, association of LVMi, the most sensitive estimate of subclinical cardiac TOD, with 24-hour urinary MBG excretion in young individuals with excessively high levels of MBG excretion.

Although our study cannot confer causality, previous work by Elkareh and colleagues^{5,6} and Kennedy and colleagues^{7,8} demonstrated an increase in the cardiac weight⁵ of rats subjected to MBG infusion, along with concurrent cardiac histological changes.^{5–8} Accordingly, the observed marked increases in collagen-1,^{5,6} fibronectin⁸ and a smooth muscle cell actin⁵ were observed in the cardiac tissue of these animals. Further evidence indicates that immunisation against MBG attenuates cardiac fibrosis.^{5,8} With no evidence from human studies, we could therefore speculate that sustained exposure to high levels of MBG may induce similar histological changes in human cardiac tissue.

Notably, the relationship between LVMI and MBG excretion was evident only in women, despite men consuming more salt¹⁵ and having a higher MBG excretion.³ Although speculative, our findings suggest that women, exhibiting lower urinary MBG excretion, may be more sensitive to the cardiac effects of MBG. Indeed, sex-specific analyses of ion channel distribution in human hearts indicated $\alpha 1\text{-Na}^+/\text{K}^+\text{-ATPase}$ to be expressed more abundantly in women compared with men.²⁷ With further limited evidence in human studies, observations from animal studies may be considered explanatory of our findings. Goel et al. previously demonstrated increased protein kinase C $\beta 2$ expression in female rats,²⁸ which promotes $\alpha 1\text{-Na}^+/\text{K}^+\text{-ATPase}$ sensitivity to MBG.²⁹ In support, the female sex hormone oestradiol was also shown to increase cardiac $\alpha 1\text{-Na}^+/\text{K}^+\text{-ATPase}$ activity in rats.³⁰ Conversely, in men, the relationships of EDVi and COi with excessively high levels of MBG excretion support a volume overload profile associated with MBG. These apparent sex differences warrant a more in-depth investigation into the relationship between MBG and sex hormones.

When studies also indicated impaired left ventricular relaxation during MBG exposure in rats,^{5,8} we found no relationships between MBG excretion and indices of left ventricular function. This suggests that the adverse effect of MBG on cardiac structure precedes that of function in young adults. Furthermore, we found no relationship between reduced kidney function and MBG excretion, possibly due to the young age of our population. However, MBG might alter kidney function at a later stage in this population consuming high amounts of salt.

Our findings should be interpreted in the context of the strengths and limitations of this study. This young study population provides a unique opportunity to gain valuable insight with regard to the possible role of MBG at an early age prior the onset of CVD. However, our cohort may not be representative of the general South African population, because we screened young adults to be apparently healthy on enrolment. Nonetheless, our findings of a positive association between LVMI and MBG in young healthy adults with excessively high levels MBG excretion may be of particular importance, as these individuals are presumably at their peak cardiovascular health. Importantly, Levy et al. reported LVMI to be a predictor of future CVD development, mortality and all-cause mortality.³¹ It is possible that the adverse effects of elevated MBG on LVMI might be aggravated during the long-term exposure to modifiable risk factors and advancing age, thereby increasing the risk of future CVD. Due to the novelty of our findings in a young cohort with elevated MBG, our work is of a hypothesis-generating nature, and we encourage replication of our findings in other populations.

Taking into consideration the aforementioned, and the levels of MBG excretion in those consuming high amounts of salt, our study supports global initiatives on sodium reduction by organisations such as the European Society of Cardiology,³² United Nations³³ and World Health Organization.³⁴

In conclusion, this is the first study demonstrating that LVMi is positively and independently associated with MBG in young apparently healthy adults with excessively high MBG excretion. These findings suggest that elevated levels of MBG, particularly in women, may increase the risk of the development of future CVD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Pearson correlations between indices of subclinical target organ damage and marinobufagenin (MBG) excretion in the total group.

Figure 2.

Left ventricular mass index of young adults across increasing quartiles of marinobufagenin (MBG) excretion. (a) Single and partially adjusted regression analyses ($\square$ Q1–Q3 and $\blacksquare$ Q4). (b) Multivariate adjusted regression analyses adjusted for age, sex, ethnicity, 24-hour systolic blood pressure, estimated glomerular filtration rate, high-density lipoprotein cholesterol, C-reactive protein, γ -glutamyl transferase and glucose.

*Comparing Q1 and Q4 adjusting for age sex and ethnicity; $P < 0.05$.

* $P < 0.05$.

Table 1.
Comparison of general participant characteristics across quartiles of MBG excretion (N = 707).

	MBG excretion quartiles					P for trend	P Q1 vs. Q4
	Q1 (N = 178)	Q2 (N = 178)	Q3 (N = 176)	Q4 (N = 179)			
Limits of quartiles (nmol/day)	0.250–2.175	2.176–3.282	3.283–5.021	5.022–12.81		<0.001	<0.001
Age (years)	25.1 ± 0.23	24.6 ± 0.23	24.8 ± 0.23	24.7 ± 0.23		0.34	0.88
Black race, N (%)	102 (57.3)	102 (57.3)	90 (51.1)	68 (38.0)		<0.001	<0.001
Men, N (%)	37 (20.8)	57 (32.0)	90 (51.1)	112 (62.6)		<0.001	<0.001
Low SES, N (%)	65 (36.5)	78 (43.8)	70 (39.8)	58 (32.4)		0.33	0.74
Anthropometric measurements							
Height (m)	1.64 ± 0.66	1.66 ± 0.66	1.69 ± 0.66	1.72 ± 0.66		<0.001	<0.001
Body mass index (kg/m ²)	26.1 ± 0.44	25.0 ± 0.44	24.9 ± 0.44	26.1 ± 0.44		0.076	0.99
Waist to height ratio	0.49 ± 0.01	0.47 ± 0.01	0.47 ± 0.01	0.49 ± 0.01		0.067	0.99
Adjusted for age, sex, ethnicity							
Cardiovascular profile							
24 hr SBP (mmHg)	116 ± 0.63	115 ± 0.62	117 ± 0.63	118 ± 0.64		0.001	<0.001
24 hr DBP (mmHg)	68.6 ± 0.43	68.1 ± 0.42	69.2 ± 0.42	69.2 ± 0.43		0.24	0.58
LVMi (g/m ²)	70.4 (68.2; 72.6)	72.0 (69.9; 74.3)	72.6 (70.4; 74.9)	74.1 (71.9; 76.5)		0.16	<0.001
Relative wall thickness (cm)	0.36 (0.35; 0.37)	0.36 (0.35; 0.38)	0.36 (0.35; 0.37)	0.36 (0.35; 0.37)		0.70	0.99
EDVi (ml/min)	59.0 (57.2; 60.8)	58.9 (57.2; 60.7)	58.9 (57.1; 60.6)	63.1 (61.2; 65.0)		0.003	<0.001
SVi (ml·min ^{-2.04})	22.7 (21.9; 23.5)	22.9 (22.2; 23.7)	22.6 (21.8; 23.3)	24.4 (23.6; 25.3)		0.003	0.004
COi (L/min ^{1.83})	1.66 (1.60; 1.73)	1.66 (1.60; 1.72)	1.64 (1.58; 1.70)	1.76 (1.70; 1.83)		0.030	0.99
Ejection fraction (%)	65.9 ± 0.47	66.5 ± 0.46	66.2 ± 0.46	67.1 ± 0.47		0.39	0.99
Fractional shortening (%)	36.4 ± 0.37	36.9 ± 0.36	36.6 ± 0.36	37.4 ± 0.37		0.35	0.99
LA:Ao	1.10 ± 0.01	1.09 ± 0.01	1.08 ± 0.01	1.09 ± 0.01		0.84	0.99
E:A	2.07 (2.00; 2.15)	2.02 (1.96; 2.10)	2.04 (1.97; 2.12)	2.07 (2.00; 2.15)		0.78	0.99
E:ε	6.33 (6.16; 6.51)	6.28 (6.12; 6.45)	6.25 (6.09; 6.42)	6.37 (6.20; 6.55)		0.78	0.98
24-Hour urinary profile and renal function							
Volume (L/24 hours)	0.99 ± 0.05	1.22 ± 0.05	1.46 ± 0.05	1.89 ± 0.05		<0.001	<0.001
MBG concentration (nmol/L)	1.56 (1.45; 1.68)	2.48 (2.31; 2.66)	3.10 (2.89; 3.33)	4.06 (3.77; 4.37)		<0.001	<0.001
MBG excretion (nmol/day)	1.41 (1.36; 1.46)	2.73 (2.63; 2.84)	4.04 (3.89; 4.19)	6.92 (6.66; 7.19)		<0.001	<0.001

	MBG excretion quartiles					P for trend	P Q1 vs. Q4
	Q1 (N = 178)	Q2 (N = 178)	Q3 (N = 176)	Q4 (N = 179)			
Na ⁺ excretion (mmol/day)	87.7 (80.8; 95.3)	116 (107; 126)	139 (132.9; 151)	201 (185; 218)	<0.001	<0.001	<0.001
MBG:Na ⁺ ratio	0.02 (0.01; 0.02)	0.02 (0.02; 0.03)	0.03 (0.03; 0.03)	0.03 (0.03; 0.04)	<0.001	<0.001	<0.001
NaCl intake (g/day)	5.17 (4.76; 5.61)	6.85 (6.33; 7.41)	8.22 (7.60; 8.90)	11.8 (10.9; 12.8)	<0.001	<0.001	<0.001
K ⁺ excretion (mmol/day)	27.2 (25.1; 29.5)	33.7 (31.2; 36.4)	43.1 (39.9; 46.6)	65.7 (60.8; 71.0)	<0.001	<0.001	<0.001
Na:K ratio	3.34 (3.09; 3.61)	3.48 (3.23; 3.75)	3.25 (3.02; 3.50)	3.15 (2.93; 3.40)	0.33	0.99	0.99
FENa (%)	0.55 (0.49; 0.61)	0.70 (0.63; 0.77)	0.80 (0.72; 0.88)	1.06 (0.96; 1.18)	<0.001	<0.001	<0.001
eGFR (ml/min/1.73m ²)	120 ± 1.26	122 ± 1.21	121 ± 1.22	120 ± 1.24	0.76	0.010	0.010
Albumin (mg/L)	3.92 (3.68; 4.19)	3.67 (3.45; 3.91)	3.89 (3.65; 4.14)	4.07 (3.82; 4.34)	0.17	0.99	0.99
Creatinine (mmol/L)	7.88 (7.30; 8.51),	8.19 (7.60; 8.82)	8.56 (7.95; 9.22)	9.54 (8.84; 10.3)	0.006	<0.001	<0.001
Biochemical profile							
Glucose (mmol/L)	4.76 ± 0.05	4.68 ± 0.05	4.71 ± 0.05	4.71 ± 0.05	0.79	0.99	0.99
HDL-C (mmol/L)	1.31 (1.25; 1.37)	1.26 (1.21; 1.31)	1.23 (1.18; 1.28)	1.22 (1.17; 1.27)	0.11	<0.001	<0.001
LDL-C (mmol/L)	2.71 (2.57; 2.86)	2.67 (2.54; 2.81)	2.58 (2.45; 2.71)	2.70 (2.57; 2.84)	0.52	0.99	0.99
C-Reactive protein (mg/L)	1.37 (1.11; 1.70)	1.02 (0.83; 1.25)	1.00 (0.81; 1.23)	0.92 (0.74; 1.13)	0.055	<0.001	<0.001
IL-6 (pg/ml)	1.06 (0.96; 1.18)	1.02(0.92; 1.14)	1.05 (0.94; 1.16)	1.01 (0.91; 1.12)	0.91	0.041	0.041
Lifestyle measures							
Cotinine > 10 (ng/ml)	209 ± 26.9	198 ± 23.7	228 ± 27.5	168 ± 23.9	0.43	0.99	0.99
γ-Glutamyl transferase (U/L)	19.3 (17.8; 21.0)	20.4 (18.8; 22.1)	23.3 (21.5; 25.2)	21.2 (19.5; 23.0)	0.018	0.033	0.033

Data presented as arithmetic mean ± SEM or geometric mean (5th and 95th percentile intervals).

Cardiovascular, renal, biochemical and lifestyle measure were adjusted for age, sex and ethnicity.

SES: socioeconomic status in total group; COi: cardiac index; DBP: diastolic blood pressure; E:A: mitral peak velocity of early filling (E) to late diastolic filling (A); EDVi: end diastolic volume index; E:é: mitral peak velocity of early filling (E) to early diastolic mitral annular velocity (é); EF: ejection fraction; eGFR: estimated glomerular filtration rate; FENa: fractional sodium excretion; FS: fractional shortening; HDL-C: High density lipoprotein cholesterol; K⁺: potassium; L:A:Ao: left atrial to aortic ratio; LVMI: left ventricular mass index; MBG: marinobufagenin; Na⁺: sodium; SBP: systolic blood pressure; SVi: stroke index.